

D16
--90 (amended). A method for preparing a trimeric polypeptide complex which comprises (i) admixing three monomer polypeptides according to claim 68, (ii) effecting complex formation between said monomer polypeptides, and (iii) isolating the resulting trimeric polypeptide complex.--

Please amend claim 94 to recite the following:

D17
--94 (amended). A chimeric product comprising a trimeric polypeptide complex according to claim 68, wherein said product does not elicit an antigenic response in a human subject.--

Please amend claim 101 to recite the following:

D18
--101 (amended). The method according to claim 100 wherein the composition is administered by a route selected from the group consisting of the intravenous route, the intraarterial route, the transmembraneus route of the buccal, anal or vaginal tissue, intranasal route, the pulmonary route, the transdermal route, intramuscular, subcutaneous, intratechal, inoculation into tissue, or by an implant.--

REMARKS

Amendments do not Introduce New Matter

No new matter is introduced into the specification by way of these amendments. New claims 106 and 107 find support throughout the specification as originally filed. New claim 106 finds support in the specification as originally filed, for example, on page 6, last paragraph extending to page 7. New claim 107 finds support in the specification as originally filed, for example, on page 34, lines 8-13 and lines 20-23. New claims 108-111 and 126-130 find support in the specification as originally filed, for example, on page 17, the last paragraph extending to page 18, line 7, and on page 35, lines 25-26. New claim 112 finds support in the specification as originally filed, for example, on page 33, lines 18-20. New claims 113-117 find support in the specification as originally filed, for example, on page 17, first full paragraph. New claim 118 finds support in the specification as originally filed, for example, on page 14, lines 11-15 and Figure 2. New claim 119 finds support in the specification as originally filed, for example, on page 8, lines 12-13 and Figure 1. New claim 120 finds support in the specification as originally filed, for example, on page 8, lines 18-20 and Figure 1. New claim 121 finds support in the specification as originally filed, for example, Figure 1. New claims 122-123 find support in the specification as originally filed,

for example, on page 21, last full paragraph. New claims 124-125 find support in the specification as originally filed, for example, page 22, lines 8-15. New claims 131-132 and 134 find support in the specification as originally filed, for example, on page 22, lines 29-33. New claim 133 finds support in the specification as originally filed, for example, on page 18, last full paragraph.

Objections

Specification

Rejections under 35 U.S.C. § 132

The Examiner objected to the amendments filed March 1, 2002 and February 2, 2001, as allegedly introducing new matter into the specification. More specifically, the Examiner objected to the language “SEQ ID NO:40 and 41 are portions of a larger Tripa DNA sequence.”

Applicants have removed the offending language from the specification by way of amendment herein. Accordingly, Applicants believe that they have fully addressed the Examiner’s concerns. Therefore, Applicants respectfully request reconsideration and withdrawal of the new matter rejection under 35 U.S.C. § 132.

Claims

The Examiner objected to claim 101 as reciting the misspelled word “og”, as well as reciting “the buccal” twice. Applicants appreciate the Examiner’s attention to these errors. Applicants have amended the claims herein, and believe that they have fully addressed the Examiner’s concerns.

Drawings

The Examiner objected to Figures 1, 3, 5, 7, and 17 in light of the respective description of each of these figures. Applicants have amended herein the descriptions of figures 1, 3, 5, 7 and 17 to address the Examiner’s objections. Applicants believe that they have addressed the Examiner’s concerns, and respectfully request reconsideration and withdrawal of the objections to Figures 1, 3, 5, 7 and 17.

Applicants further submit herewith proposed amendments to Figures 1, 2, 3, 5, 6, 7, 13, 15, 17 and 19 under 37 C.F.R. § 1.121. The proposed amendments to the figures are presented in red ink on a separate sheet for each respective figure, for approval by the

Examiner. Applicants respectfully request consideration of the proposed amendments to the Figures and notification of acceptance of the Figure amendments by the Examiner.

Rejections

Rejections under 35 U.S.C. § 112, 2nd Paragraph

The Examiner rejected claims 22-24, 76, 82, 84, 87-90 and 94 under 35 U.S.C. § 112, 2nd Paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicants have either amended or cancelled the rejected claims under the claim amendments presented *supra*, and accordingly have addressed the Examiner's rejections. Applicants respectfully request reconsideration and withdrawal of the rejections of claims 22-24, 76, 82, 84, 87-90 and 94 under 35 U.S.C. § 112, 2nd Paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Rejections under 35 U.S.C. § 102

The Examiner rejected claims 1, 19, 68-77, 83, 86, 90 and 98 under 35 U.S.C. § 102(b) as allegedly anticipated by Thogersen *et al* as evidenced by Kastrup *et al*, and as evidenced by the abstract of Nielsen *et al*. More specifically, the Examiner asserts that "Thogersen *et al* disclose the sequence for human tetranectin comprising the consensus sequence of Figure 2 and comprising residues E1 to K52, and a peptide construct pT7H6FX-TETN (page 91, lines 20-21) expressing the tetranectin monomer linked via a peptide bond at the N-terminus (page 91, lines 10 to 18 indicate fusion of the SEQ ID NO:37 to the tetranectin Glu1 residue) to heterologous sequence encoding a cleavage site for the bovine restriction protease." See Paper No. 16, Page 10, lines 14-18. Additionally, the Examiner states that

it can be concluded that the oligomer construct of Thogersen *et al* is a trimer, said trimer comprising 3 heterologous moieties, as each tetranectin molecule in the trimer is fused to the heterologous protein. The trimeric oligomer construct exists as a triple alpha helical coiled-coil complex. This physical state is an inherent property of the tetranectin trimer.

Accordingly, the Examiner rejected claims 1, 19, 68-77, 83, 86, 90 and 98 under 35 U.S.C. § 102(b) as allegedly anticipated by Thogersen *et al* as evidenced by Kastrup *et al*, and as evidenced by the abstract of Nielsen *et al*.

In light of the amendments to Claim 1, Applicants respectfully disagree and traverse this rejection.

In order for a reference to anticipate under 35 U.S.C. § 102(b), the reference must disclose all limitations of the claimed invention in a printed publication in this or a foreign country more than one year prior to the date of application for patent in the United States. *See MPEP* § 2133. Thus in order for Thogersen *et al.*, as evidenced by Kastrup *et al.* and Nielsen *et al.*, to be proper 35 U.S.C. § 102(b) art, all elements of the claimed invention must be described in a printed publication in this or a foreign country more than one year prior to the date of application for patent in the United States.

Thogersen *et al* fails this test.

As amended herein, pending claim 1 recites:

1. A monomer polypeptide construct comprising at least one tetranectin trimerising structural element (TTSE) which is covalently linked to at least one heterologous moiety, said TTSE being capable of forming a stable triple alpha helical coiled coil complex with two other TTSEs, where the heterologous moiety is different from any of the fusion proteins CIIH6FXTN123, H6FXTN123, H6FXTN12, H6FCTN23, the sequences of which are shown in SEQ ID NOs:24-27.

Thogersen *et al* disclose “the sequence for human tetranectin comprising the consensus sequence of Figure 2 and comprising residues E1 to K52, and a peptide construct pT7H6FX-TETN (page 91, lines 20-21) expressing the tetranectin monomer linked via a peptide bond at the N-terminus (page 91, lines 10 to 18 indicate fusion of the SEQ ID NO:37 to the tetranectin Glu1 residue) to heterologous sequence encoding a cleavage site for the bovine restriction protease.” *See* Paper No. 16, Page 10, lines 14-18. The polypeptide produced by Example 9 of WO 94/18227 results in the sequence represented in the instant application as SEQ ID NO:25, which correlates to fusion protein H6FTXN123. As recited in amended claim 1, the claimed heterologous moiety covalently linked to the monomer polypeptide construct “is different from any of the fusion proteins ... H6FTXN123.” The fusion protein in Thogersen *et al.* cited by the Examiner as the basis for the 35 U.S.C. § 102(b) rejection produces the fusion protein H6FTXN123. Fusion protein H6FXTN123 is specifically disclaimed from the subject matter of pending claim 1. As a result, International Publication No. WO 94/18227 is not available as proper § 102(b) prior art, as this reference does not meet all of the limitations of claim 1. Claims 19, 74, 75 and 76 ultimately incorporate all of the limitations of claim 1 through dependency. Therefore, International

Publication No. WO94/18227 is equally inapplicable as proper § 102(b) prior art against claims 19, 74, 75 and 76.

Similarly, claim 68 as amended herein includes the specific limitation that “said heterologous moiety is different from any of the fusion proteins CIIH6FXTN123, H6FXTN123, H6FXTN12, H6FCTN23, the sequences of which are shown in SEQ ID NOs:24-27.” For the reasons set forth above, the fusion protein of Thogersen *et al.* cited by the Examiner as the basis for the 35 U.S.C. § 102(b) rejection, produces the fusion protein H6FTXN123 which is specifically excluded from the scope of amended claim 68.

As a result, Thogersen *et al.* is equally inapplicable as proper § 102(b) prior art to claims 69-73, 77, 83, 86, 90 and 98, which depend from claim 68.

Accordingly, Thogersen *et al.*, as evidenced by Kastrup *et al.* and Nielsen *et al.*, does not disclose all of the limitations of the claimed invention, and therefore is not available as proper 35 U.S.C. § 102(b) art against the instant invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejections of claims 1, 19, 68-77, 83, 86, 90 and 98 under 35 U.S.C. § 102(b) as allegedly anticipated by Thogersen *et al.* as evidenced by Kastrup *et al.* and Nielsen *et al.*.

Rejections under 35 U.S.C. § 103

The Examiner rejected claims 22, 23, 68-77, 82-90, 94, 98, 99, 102 and 103 under 35 U.S.C. § 103(a) as allegedly unpatentable over Thogersen *et al.* and Kastrup *et al.*, in view of Hoppe *et al.* More specifically, the Examiner asserts that “Thogersen *et al.*, as evidenced by Kastrup *et al.*, teach that tetranectin forms trimers in solution and in the crystal state.” *See* Paper No. 16, Page 14, lines 4-5. Furthermore, the Examiner states that Hoppe *et al.* teach “trimerizing polypeptides comprising heterologous moieties attached N-terminally or C-terminally or both N and C terminally to a collectin-neck region polypeptide, said neck-region polypeptide able to form a trimer and the trimeric polypeptide complex comprising at least 2, 3, 4, 5 or 6 heterologous moieties.” *See* Paper No. 16, Page 15, lines 2-5. The Examiner concludes that “[i]t would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the tetranectin monomer for the collectin neck region peptide disclosed by Hoppe *et al.* and symbolized by the structure on page 3, lines 11-16.” *See* Paper 16, Page 16, lines 3-7. The Examiner further states that “substitution of the tetranectin monomer for the collectin neck region peptide would result in a monomer having the ability to trimerize as the collectin neck region peptide.” *See* Paper 16, Page 16, lines 16-18.

Applicants respectfully disagree and traverse this rejection.

As stated by the Federal Circuit, “a proper analysis under 35 U.S.C. § 103 requires, *inter alia*, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success.” *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991). In addition, the prior art reference(s) must teach or suggest all of the claim limitations. The teaching or suggestion to combine and the reasonable expectation of success must both be found in the prior art, and not in Applicants’ disclosure. *Id* at 493. *See also* M.P.E.P. § 2142. In order to find such motivation or suggestion, there should be a reasonable likelihood that the claimed invention would have the properties disclosed by the prior art teachings. *See* M.P.E.P. § 2144.08(II)(A).

In the present case there is no motivation, suggestion or teaching to combine the references to arrive at the claimed invention. Neither the Thogersen *et al* reference, the Kastrup *et al* reference, or the Hoppe *et al* reference provides the requisite motivation, suggestion or teaching to those of ordinary skill in the art to combine the Thogersen *et al* reference, the Kastrup *et al* reference, and the Hoppe *et al* reference to arrive at Applicants’ claimed invention.

In the present case, the Examiner has done no more than find the separate elements of the present invention and argue that broad disclosures which would require specific selection and experimentation to achieve the current invention, render the present invention obvious. The fact that each element of an invention may be found somewhere in the prior art is unavailing. A combination may be patentable whether it be composed of elements all new, partly new or all old. *Rosemont, Inc. v. Beckman Instruments*, 221 USPQ 1, 7 (Fed. Cir. 1994).

In addition, it is of no doubt that one of ordinary skill in the art, enlightened by Applicants’ teachings, would be motivated “with a reasonable expectation of success.” *See* Paper 3, Page 16, lines 6-7. The issue remains, however, whether the necessary teaching, suggestion or motivation to combine the Thogersen *et al* reference, the Kastrup *et al* reference, and the Hoppe *et al* reference to arrive at Applicants’ claimed invention was provided by the references themselves, and not Applicants’ teachings. No proper teaching, suggestion or motivation to combine said references is provided by the Thogersen *et al* reference, the Kastrup *et al* reference, and the Hoppe *et al* reference, and accordingly,

Applicants' invention as claimed is not rendered obvious in light of the individual references or the combination thereof.

Notwithstanding a lack of motivation to combine the Thogersen *et al* reference, the Kastrup *et al* reference, and the Hoppe *et al* reference, a *prima facie* case of obviousness based on structural similarity is rebuttable by proof that the claimed compounds possess unexpectedly advantageous or superior properties. As stated in the M.P.E.P., “[e]vidence of unobvious or unexpected advantageous properties, such as superiority in a property the claimed compound shares with the prior art, can rebut *prima facie* obviousness. ‘Evidence that a compound is unexpectedly superior in one of a spectrum of common properties...can be enough to rebut a *prima facie* case of obviousness.’ No set number of examples of superiority is required.” See M.P.E.P. § 716.02(a).

As explicitly recognized by the inventors of the instant application, “[t]he thermal stability of the tetranectin trimerisation module (as shown in the examples) is such that the trimer can be shown to exist even at about 60°C (Example 4, trimerised tetranectin) or at about 70°C (trimerized ubiquitin), whereas a collectin trimer unit falls apart at about 50-55°C (WO 95/31540, Example 1, page 36 therein).” See specification, page 6, first full paragraph. Accordingly, it is clearly seen that the thermal stability of the tetranectin trimerisation module of the present invention is significantly higher than the thermal stability of the prior art collectin-neck region trimeric polypeptide described by Hoppe *et al.* in WO 95/31540, which falls apart at about 50-55°C.

This surprising high thermal stability, as compared to the prior art collectin trimer, is further seen from the specification where it is stated that “a substantial part of the recombinant proteins exists in the oligomeric state of - and can be cross-linked as - trimeric molecules even at 70°C”, (See specification, Page 15, lines 32-34); “SDS-PAGE analysis of reduced samples (Fig. 12) showed, that trimers are readily detectable even at 60°C”, (See specification, Page 49, lines 35-37); and “...a substantial amount of trimer molecules is present even at 70°C” (See specification, Page 51, lines 33-34).

This improvement is surprising and unexpected in light of the prior art, and is significant. Assuming, *arguendo*, that it would be *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the tetranectin monomer for the collectin neck region peptide disclosed by Hoppe *et al.*, which it would not have been, one of ordinary skill in the art would expect to synthesize a trimeric polypeptide complex with a thermal stability having an upper limit of approximately 50-55°C. Contrary to the expectations of one of ordinary skill in the art, the inventors of the subject matter of the

instant application have generated trimeric polypeptides which are thermally stable, even at temperatures as high as 70°C in certain embodiments. This thermal stability is an extremely useful and unexpected trait that enables the provision of trimeric polypeptide carrier molecules having a high thermal stability, which do not disintegrate into monomer subunits even at relatively high temperatures. Consequently, by having this high thermal stability, the trimeric polypeptide molecules according to the invention would have no, or very few occurrences of exchange of monomer subunits between different trimeric polypeptide molecules, and hence the high thermal stability implies improved shelf life of the trimeric polypeptide molecules.

There are no teachings in association with trimerising structural elements derived from collectin which teach or suggest high thermal stability and improved shelf life on the order discovered by the inventors of the instant application.

These unexpected properties, disclosed by the Applicants in the priority filing, are substantial evidence that the invention as claimed was not *prima facie* obvious to one of ordinary skill in the art at the time of filing. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejections of claims 22, 23, 68-77, 82-90, 94, 98, 99, 102 and 103 under 35 U.S.C. § 103(a) as allegedly unpatentable over Thogersen *et al.* and Kastrup *et al.*, in view of Hoppe *et al.*.

The Examiner rejected claims 22, 23, 68-77, 82-91, 94, 102 and 103 under 35 U.S.C. § 103(a) as allegedly unpatentable over Thogersen *et al.*, Kastrup *et al.* and the abstract of Nielsen *et al* in view of Hoppe *et al.* and Baker *et al.* More specifically, the Examiner asserts that “[i]t would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the diagnostic agent comprising the trimeric polypeptide complex of tetranectin linked to an antibody and a detectable label as taught by the combination of Thogersen *et al.*, Kastrup *et al.*, and Nielsen *et al* and Hoppe *et al* for the anti-cardiotrophin-1 antibody, detectable label and kit comprising said antibody as taught by Baker *et al.*.”

Applicants respectfully disagree and traverse this rejection.

As stated by the Federal Circuit, “a proper analysis under 35 U.S.C. § 103 requires, *inter alia*, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of

success." *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991). In addition, the prior art reference(s) must teach or suggest all of the claim limitations. The teaching or suggestion to combine and the reasonable expectation of success must both be found in the prior art, and not in Applicants' disclosure. *Id* at 493. *See also* M.P.E.P. § 2142. In order to find such motivation or suggestion, there should be a reasonable likelihood that the claimed invention would have the properties disclosed by the prior art teachings. *See* M.P.E.P. § 2144.08(II)(A).

In the present case there is no motivation, suggestion or teaching to combine the references to arrive at the claimed invention. Neither the Thogersen *et al* reference, the Kastrup *et al* reference, the Hoppe *et al* reference or the Baker *et al* reference provides the requisite motivation, suggestion or teaching to those of ordinary skill in the art to combine the Thogersen *et al* reference, the Kastrup *et al* reference, the Hoppe *et al* reference and the Baker *et al* reference to arrive at Applicants' claimed invention.

In the present case, the Examiner has done no more than find the separate elements of the present invention and argue that broad disclosures which would require specific selection and experimentation to achieve the current invention, render the present invention obvious. The fact that each element of an invention may be found somewhere in the prior art is unavailing. A combination may be patentable whether it be composed of elements all new, partly new or all old. *Rosemont, Inc. v. Beckman Instruments*, 221 USPQ 1, 7 (Fed. Cir. 1994).

In view of the unexpected results discussed above, Applicants claimed invention was not obvious to one of ordinary skill in this art. More specifically, "[t]he thermal stability of the tetranectin trimerisation module (as shown in the examples) is such that the trimer can be shown to exist even at about 60°C (Example 4, trimerised tetranectin) or at about 70°C (trimerized ubiquitin), whereas a collectin trimer unit falls apart at about 50-55°C (WO 95/31540, Example 1, page 36 therein)." *See* specification, page 6, first full paragraph.

As discussed above, this improvement is surprising and unexpected in light of the prior art, and is significant. With a high thermal stability, trimeric polypeptides comprising the tetranectin trimerisation module has increased stability at elevated temperature (shown to exist even at 70°C) and increased shelf life. Accordingly, the trimeric polypeptides comprising the tetranectin trimerisation module are very useful as alternative trimeric polypeptide carrier molecules to be used in, e.g., protein library technology, diagnostic applications, and in a particularly preferred embodiment of the invention as therapeutic systems, such as pharmaceutical compositions.

These unexpected properties, disclosed in the priority filing, are substantial evidence that the invention as claimed was not *prima facie* obvious to one of ordinary skill in the art at the time of filing. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejections of claims 22, 23, 68-77, 82-91, 94, 102 and 103 under 35 U.S.C. § 103(a) as allegedly unpatentable over Thogersen *et al.*, Kastrup *et al.* and the abstract of Nielsen *et al* in view of Hoppe *et al.* and Baker *et al.*

Conclusion

Applicants believe that incorporation of the amendments and consideration of the above remarks have placed this application in a condition for allowance. Early notification of a favorable consideration is respectfully requested.

To the extent necessary, the Commissioner is authorized to grant any extension of time deemed needed for entry of this document and to charge additional fees associated with this communication or such extension of time or credit any overpayment to Deposit Account No. 50-0206.

Respectfully submitted,

Dated: December 2, 2002

By:



Stanislaus Aksman
Registration No. 28,562
Robert C. Lampe, III
Registration No. 51,914

HUNTON & WILLIAMS
1900 K Street, N.W.
Suite 1200
Washington, D.C. 20006-1109
Telephone: (202) 955-1928
Facsimile: (202) 778-2201



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number: 09/445,576

Examiner: Canella, K.

Filing Date: July 17, 2000

Art Unit: 1642

Title: Trimerising Module

Inventor: Thogersen, H., et al

Commissioner of Patents and Trademarks

Washington, D.C., 20231

APPENDIX A - Version with Markings to show Changes Made to the Specification

At Page 9, lines 3-11:

--Figure 3 depicts the construction of the expression plasmids pTH6FXtripa and pTH6FXtripb. Following the teachings of Example 1, tetranectin fusion proteins H6FXtripa (SEQ ID NO:28) and H6FXtripb (SEQ ID NO:29) are produced. To generate expression plasmids pTH6FXtripa and pTH6FXtripb, the polynucleotides encoding tetranectin fragments are The amplified DNA fragments tripa (SEQ ID NO:40, bases 3-41 of which encode for residues 11-23 of SEQ ID NO:28, and SEQ ID NO:41 which encodes for residues 67-73 of SEQ ID NO:28) and tripb (SEQ ID NO:40, bases 3-41 of which encode for residues 11-23 of SEQ ID NO:28, while bases 1 and 2 are part of the codon for residue 10 of SEQ ID NO:28; and SEQ ID NO:42, which encodes for residues 64-69 of SEQ ID NO:29) harboring the tetranectin amino acid sequence (SEQ ID NO:7) from E1 to T48 and E1 to K52, respectively, fused in the 5' end to nucleotide sequences encoding FX_a cleavage site IQGR (residues 3-6 of SEQ ID NO:4103) and the recognition sites for the restriction endonucleases BglII and KpnI, were cut with the restriction enzymes BclI and HindIII and ligated into the BamHI (SEQ ID NO:43 which encodes for residues 1-9 of SEQ ID NO:28) and HindIII (SEQ ID NO:44) sites of the expression plasmid pT7H6 (Christensen et al., 1991) using standard techniques. SEQ ID NOs: 40 and 41 are portions of a larger tripa DNA sequence; the dashes denote the unquoted portion of the DNA sequence. Likewise, SEQ ID NOs: 40 and 42 are portions of the tripb DNA sequence, and SEQ ID NOs: 43 and 44, of an unnamed DNA sequence.--

At Page 9, lines 18-30:

--Figure 5 depicts the generation of expression plasmids pTH6FXTN123 and pT_CI_H6FXTN123. The amplified DNA fragment corresponding to the full length, encoding the mature tetranectin monomer (SEQ ID NO:7) from E1 to V181 protein fragment is fused in the 5' end to nucleotide sequences encoding a FX_a cleavage site IEGR (residues 3-6 of SEQ ID NO:10-104). As is apparent from the teachings of Example 2, the N- and C-terminal residues of the mature tetranectin protein fragment are represented in Figure 5 as contained within amino acid fragments SEQ ID NO: 77 and SEQ ID NO: 78, respectively. This DNA was further cut with the restriction enzymes BamHI and HindIII and ligated into the corresponding sites of the expression plasmids pT7H6 (Christensen et al., 1991) and pT_CI_H6 using standard procedures. pT_CI_H6 was derived from pT7H6 by substitution of the NdeI-HindIII fragment of pT7H6 with the NdeI-HindIII fragment of pLcII (as disclosed by Nagai and Thogersen, 1987), encoding the first 32 residues of the lambda cII protein MVRANKRNEALRIESALLNKIAMLGTEKTAEG (SEQ ID NO:11) fused in the 3' end to a nucleotide sequence encoding the H6 sequence GSHHHHHHGS (SEQ ID NO:12). In Fig. 5 the first sequence is a modified SEQ ID NO:7. The "EP" (Gly Pro) corresponds to amino acids 1-2 of SEQ ID NO:7, and the "IV" (Ile Val) to amino acids 180-181 of SEQ ID NO:7. The "—" corresponds to unquoted (but still present) DNA encoding amino acids 3-179 of SEQ ID NO:7. The sequence "GSIEGRGEP" (Gly Ser Ile Glu Gly Arg Gly Glu Pro) corresponds to amino acids 41-49 of SEQ ID NO:24, and the recited coding sequence for this nonpeptide is SEQ ID NO:45. The "—" denotes the unquoted DNA sequence joining SEQ ID NO:45 to the "ATCGTGTA" sequence.

In the next set of sequences, the "MVRA" (Met Val Arg Ala) corresponds to amino acids 1-4 of SEQ ID NO:24, and is encoded by SEQ ID NO:46. The "EGGSHHHHHH" (Glu Gly Gly Ser His His His His His) corresponds to amino acids 31-40 of SEQ ID NO:24 and is encoded by SEQ ID NO:47. The "AGCTTGAATTC" is SEQ ID NO:48. The dashes denote unquoted DNA joining SEQ ID NO:46 to SEQ ID NO:47, and SEQ ID NO:47 to SEQ ID NO:48.

— In the third set of sequences, the identifications are the same as for the first set.

— In the fourth set of sequences, the "MGSHHHHHH" (Met Gly Ser His His His His His His) corresponds to amino acids 1-9 of SEQ ID NOS: 28 and 29, and is encoded by SEQ ID NO:43.—

At Page 10, lines 3-16:

--Figure 7 depicts the generation of expression plasmids pTH6FXTN12, pTH6FXTN23 and pTH6FXTN3. Amplified DNA fragments encoding tetranectin protein fragments are fused at their 5' end to nucleotide sequences encoding a FX_a cleavage site IEGR (SEQ ID NO: 104). These DNA fragments were further cut with the restriction enzymes BamHI and HindIII, and ligated into the corresponding sites of the expression plasmid pT7H6 (Christensen et al., 1991) using standard procedures. The resulting fusion proteins are represented by SEQ ID NOs: 26, 27 and 30, respectively. As is apparent from the teachings of Example 2, the N- and C-terminal residues of the tetranectin protein fragments contained within fusion proteins 26, 27 and 30 are represented in Figure 7 as contained within amino acid fragments SEQ ID NO: 82 and SEQ ID NO: 83, amino acid fragments SEQ ID NO: 84 and SEQ ID NO: 85, and amino acid fragments SEQ ID NO: 86 and SEQ ID NO: 85, respectively. The amplified DNA fragments corresponding to the tetranectin derivatives TN12 (SEQ ID NO: 49 which encodes for residues 10-19 of SEQ ID NO: 26 and SEQ ID NO: 50) and TN3 from E1 to V49 and A45 to V181, respectively (SEQ ID NO: 7) fused in the 5' end to nucleotide sequences encoding the FX_a cleavage site IEGR (residues 3-6 of SEQ ID NO: 10) was cut with the restriction enzymes BamHI and HindIII and ligated into the corresponding sites of the expression plasmids pT7H6 (Christensen et al., 1991) using standard procedures. The amplified DNA fragment corresponding to the tetranectin derivative TN23 from V17 to V181 (SEQ ID NO: 7) fused in the 5' end to nucleotide sequences encoding the FX_a cleavage site IQGR (residues 3-6 of SEQ ID NO: 4) was cut with the restriction enzymes BamHI and HindIII and ligated into the corresponding sites of the expression plasmids pT7H6 (Christensen et al., 1991) using standard procedures.

In Fig. 7, in the line labeled "TN12", SEQ ID NO: 49 encodes amino acids 10-19 of SEQ ID NO: 26. Amino acids 17-19 of SEQ ID NO: 26 correspond to amino acids 1-3 of SEQ ID NO: 7. The "QTV" (Gln-Thr-Val) correspond to amino acids 47-49 of SEQ ID NO: 7 and are encoded by SEQ ID NO: 50.

In the line labeled "TN23", SEQ ID NO: 51 encodes amino acids 10-19 of SEQ ID NO: 27, and amino acids 16-19 of SEQ ID NO: 27 correspond to amino acids 17-20 of SEQ ID NO: 7. The "GIV" (Gly-Ile-Val) correspond to amino acids 179-181 of SEQ ID NO: 7 and are encoded by SEQ ID NO: 52.

~~In the line labeled "TN3", SEQ ID NO:53 encodes amino acids 10-19 of SEQ ID NO:30. Amino acids 17-19 of SEQ ID NO:30 correspond to amino acids 45-47 of SEQ ID NO:7 and are encoded by SEQ ID NO:54.~~

~~In the unlabeled line, SEQ ID NO:43 encodes amino acids 1-9 of SEQ ID NO:30, and the AGCTTGAATTC is SEQ ID NO:44.~~

At Page 12, between lines 8 and 9:

~~--In Fig. 13, the sequence "GSQIFV" (Gly Ser Gln Ile Phe Val) corresponds to amino acids 68-73 of SEQ ID NO:31, and is encoded by SEQ ID NO:55. The "RGGS" (Arg Gly Gly Ser) corresponds to amino acids 142-145 of SEQ ID NO:31, and is encoded by SEQ ID NO:56. For other sequences, see the description of Figure 3, but note that the "GAATTC" are bases 6-11 of SEQ ID NO:44.~~

At Page 12, between lines 20 and 21:

~~--In Fig. 15, "SQVQL" (Ser Gln Val Gln Leu) correspond to amino acids 16-20 of SEQ ID NO:32, and are encoded by SEQ ID NO:57. "LNGA" (Leu Asn Gly Ala) correspond to amino acids 275-278 of SEQ ID NO:32, and are encoded by SEQ ID NO:58. In the second set, the first amino acid sequence corresponds to amino acids 1-23 of SEQ ID NO:33 and is encoded by SEQ ID NO:59, and the second to amino acids 64-69 of SEQ ID NO:## and is encoded by SEQ ID NO:60 (which also includes SEQ ID NO:42, compare Figure 3).~~

At Page 12, lines 26-32:

~~--The DNA fragment, amplified with the primer pairs having SEQ ID NO:21 and 23, comprising the nucleotide sequence (SEQ ID NO:20) encoding the single chain antibody CEA6, scFv (CEA6), amino acid sequence from Q1 to A261 was cut with the restriction enzymes BamHI and HindIII and ligated into the BamHI and HindIII sites of the expression plasmid pT7H6FXtripb (Example 1) using standard procedures. The sequences of Fig. 17 were identified above in connection with Fig. 15.~~

At Page 13, between lines 12 and 13:

~~--The sequences of Fig. 19 were identified above in connection with Fig. 15. Generally, in Figures 13, 15, 17, and 19, dashes denote unquoted DNA sequences.~~

At Page 15, line 30 extending to page 16, line 16:

--The TTSEs form surprisingly stable trimeric molecules (Examples 2, 3, and 4). The experimental observations, that (1) a substantial part of the recombinant proteins exists in the oligomeric state of, - and can be cross-linked as, - trimeric molecules even at 70°C and (2) that exchange of monomers between different trimers can only be detected after exposure to elevated temperature are evidence of a extremely high stability of the tetranectin trimerising structural element. This feature must be reflected in the amino acid sequence of the structural element. In particular, the presence and position of the glutamine containing repeat in the sequential array of heptad repeats is, together with the presence and relative position of the other conserved residues in the consensus sequence (Fig. 2), considered important for the formation of these stable trimeric molecules. For most practical uses the cysteine residue 50 should be mutagenized to serine, threonine, methionine or to any other amino acid residue in order to avoid formation of an unwanted inter-chain disulphide bridge, which eventually would lead to uncontrolled multimerisation, aggregation and precipitation of a polypeptide product harbouring this sequence.--



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number: 09/445,576

Examiner: Canella, K.

Filing Date: July 17, 2000

Art Unit: 1642

Title: Trimerising Module

Inventor: Thogersen, H., et al

Commissioner of Patents and Trademarks

Washington, D.C., 20231

APPENDIX B - Version with Markings to show Changes Made to the Claims

1 (twice amended). A monomer polypeptide construct comprising at least one tetranectin trimerising structural element (TTSE) which is covalently linked to at least one heterologous moiety, said TTSE being capable of forming a stable triple alpha helical coiled coil complex with two other TTSEs, where (a) the heterologous moiety is different from any of the fusion proteins CIIH6FXTN123, H6FXTN123, H6FXTN12M, H6FCTN23, the sequences of which are shown in SEQ ID NOs:24-27; and/or (b) ~~at least one heterologous moiety being one in which does not exclusively facilitate expression and/or purification of the monomer polypeptide construct.~~

22 (twice amended). A trimeric polypeptide complex according to claim 68, wherein the at least one heterologous moiety which is positioned N-terminally to a TTSE and the at least one heterologous moiety which is positioned C-terminally to a TTSE are part of the same monomer polypeptide.

23 (twice amended). A trimeric polypeptide complex according to claim 68, wherein the at least one heterologous moiety which is positioned N-terminally to a TTSE and the at least one heterologous moiety which is positioned C-terminally to a TTSE are part of two separate monomer polypeptides.

68 (amended). A trimeric polypeptide complex comprising three monomer polypeptides, wherein (i) each of said monomer polypeptides comprises a tetranectin trimerising structural element (TTSE), said TTSE being a polypeptide having at least 68% amino acid sequence identity with the consensus sequence shown in Fig. 2, and (ii) at least one of said monomer polypeptides is covalently linked to at least one heterologous moiety, where said at least one heterologous moiety is different from any of the fusion proteins C11H6FXTN123, H6FXTN123, H6FXTN12, H6FCTN23, the sequences of which are shown in SEQ ID NOs:24-27.

76 (amended). The trimeric polypeptide complex according to claim 75, wherein the TTSE derived from human tetranectin further comprises the amino acid residues C50 to K52 ~~(exon 3) of exon 3 as~~ shown in Figure 1.

82 (amended). The trimeric polypeptide complex according to claim 68 ~~which is stable wherein the complex remains substantially as a trimer~~ in the temperature range 50-70°C.

84 (amended). The trimeric polypeptide complex according to claim 68, wherein the at least one heterologous moiety is selected from the group consisting of ~~a ligand binding structure; a toxin; a detectable label; an in situ activatable substance; an enzyme; a radioactive moiety; a cytokine; a non proteinaceous polymer such as a polymeric alkaloid, a polyalcohol, a polysaccharide, a lipid and a polyamine; a photo cross linking agent; and a group facilitating conjugation of the polypeptide to a target.~~:

- (a) a ligand binding structure;
- (b) a toxin;
- (c) a detectable moiety;
- (d) an in situ activatable substance;
- (e) an enzyme;
- (f) a radioactive moiety;
- (g) a cytokine;
- (h) a non-proteinaceous polymer;
- (i) a polyalcohol;
- (j) a polysaccharide;
- (k) a lipid;
- (l) a polyamine;

(m) a photo cross-linking agent; and

(n) a group facilitating conjugation of the polypeptide to a target, wherein the conjugation encompasses both covalent and non-covalent linkages.

87 (amended). The trimeric polypeptide complex according to claim 68, which comprises at least one heterologous moiety which is positioned N-terminally to ~~the at least one~~ monomer polypeptide and at least one heterologous moiety which is positioned C-terminally to ~~the at least one~~ monomer polypeptide.

88 (amended). The trimeric polypeptide complex according to claim 68, wherein the at least one heterologous moiety is covalently linked to the monomer polypeptide via a peptide bond to the N- or C-terminus of the monomer polypeptide chain, via a peptide bond to a side chain in the monomer polypeptide, via a bond to a cysteine residue, or when more than one heterologous moiety, combinations of these locations.

90 (amended). A method for preparing a trimeric polypeptide complex which comprises (i) admixing three monomer polypeptides according to claim 68, (ii) effecting complex formation between said monomer polypeptides, and (iii) isolating the resulting trimeric polypeptide complex ~~and optionally subjecting the polypeptide complex to further processing.~~

94 (amended). A chimeric product comprising a trimeric polypeptide complex according to claim 68, wherein said product ~~having low antigenicity in humans relative to formulations comprising one or more components of non-human origin~~ does not elicit an antigenic response in a human subject.

101 (amended). The method according to claim 100 wherein the composition is administered by a route selected from the group consisting of the intravenous route, the intraarterial route, the transmembraneus route of the buccal, anal ~~or~~ vaginal tissue, intranasal route, the pulmonary route, the transdermal route, intramuscular, subcutaneous, intratechal, ~~the buccal,~~ inoculation into tissue, or by an implant.

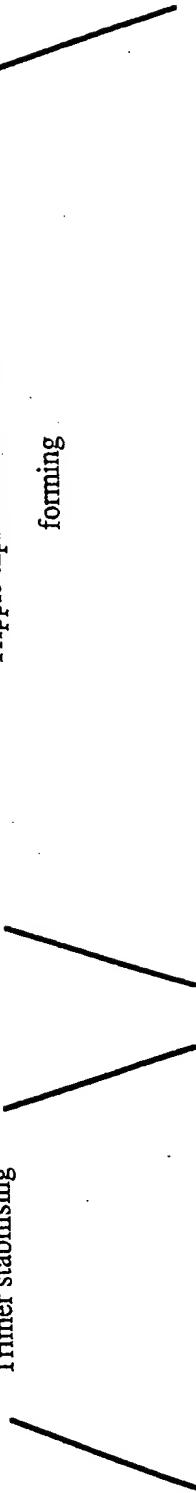


WO 98/56906

PCT/DK98/00245

1/21

Exon 1
Trimer stabilising
Exon 2
Triple alpha helical coiled coil
forming



E1P2P T Q K P K I V N A K K D16 V17V N T K M F E E L K S R L D T L A Q E V A L L K E Q Q A L Q T V C L S K S2
SEQ ID NO: 35
71

Fig. 1



WO 98/56906

PCT/DK98/00245

2/21

Fig. 2



WO 98/56906

DEC 02 2002

PCT/DK98/00245

tripa

SEQ ID NO: 72
G S I Q G R S P G T E P P T
GATCAAATCCAGGGAAAGATCTCTGGTACCGAGCCACCAACC -----
Bgl II Kpn II SEQ ID NO: 41

SEQ ID NO: 73
G S I Q G R S P G T E P P T
GATCAAATCCAGGGAAAGATCTCTGGTACCGAGCCACCAACC -----
Bgl II Kpn II SEQ ID NO: 42

SEQ ID NO: 74
G S I Q G R S P G T E P P T
GATCAAATCCAGGGAAAGATCTCTGGTACCGAGCCACCAACC -----
Bgl II Kpn II SEQ ID NO: 43

3/21

SEQ ID NO: 75
G S I Q G R S P G T E P P T
GATCAAATCCAGGGAAAGATCTCTGGTACCGAGCCACCAACC -----
Bam HI SEQ ID NO: 42

SEQ ID NO: 76
M G S H H H H H H H H
CATATGGGATCGCATCACCATCACCATCACG ----- AGCTTGAAATT
SEQ ID NO: 45

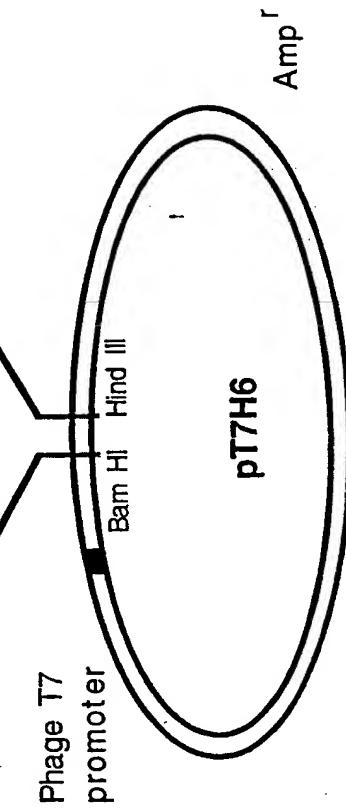


Fig. 3



WO 98/56906

PCT/DK98/00245

5/21

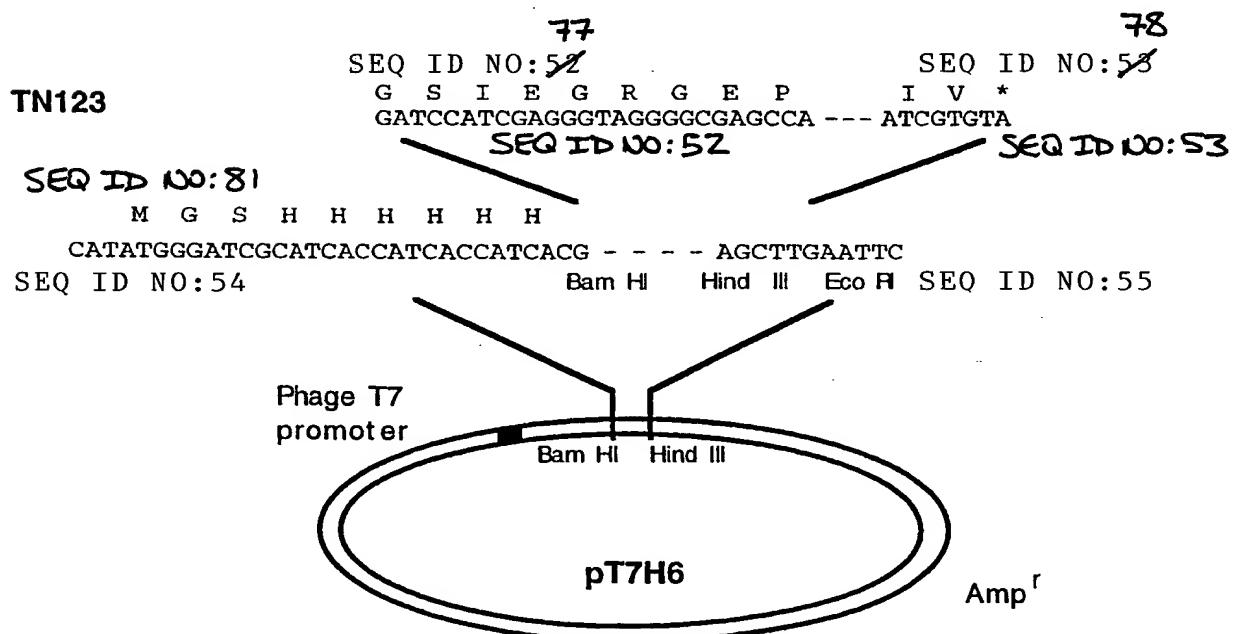
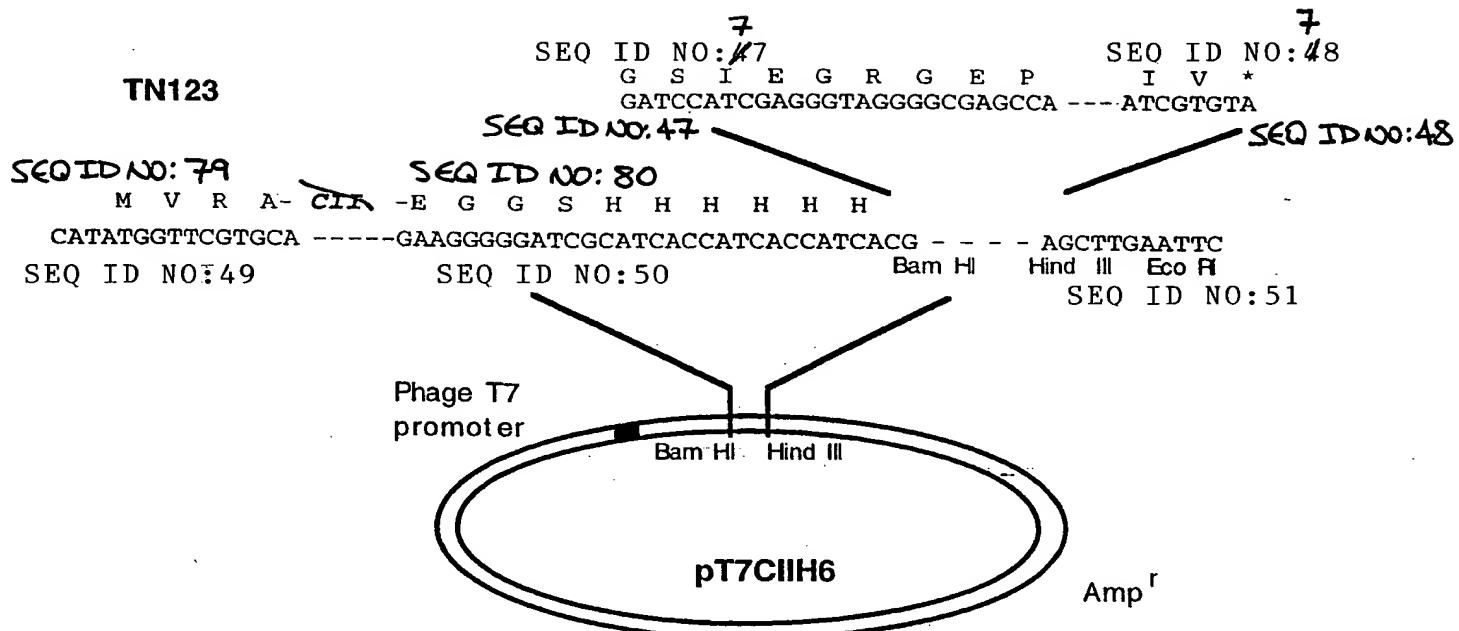


Fig. 5



98/56906

DEC 02 2002

PCT/DK98/00245

6/21

CIIH6FXTN123 fusion protein SEQ ID NO: 24

1 M V R A N K R N E A L R I E S A L L N K I A M L G T E K T A 30
31 E G G S H H H H H H G S I E G R G E P P T Q K P K K I V N A 60
61 K K D V V N T K M F E E L K S R L D T L A Q E V A L L K E Q 90
91 Q A L Q T V C L K G T K V H M K C F L A F T Q T K T F H E A 120
121 S E D C I S R G G T L S T P Q T G S E N D A L Y E Y L R Q S 150
151 V G N E A E I W L G L N D M A A E G T W V D M T G A R I A Y 180
181 K N W E T E I T A Q P D G G K T E N C A V L S G A A N G K W 210
211 F D K R C R D Q L P Y I C Q F G I V * 228

H6FXTN123 fusion protein SEQ ID NO: 25

1 M G S H H H H H H G S I E G R G E P P T Q K P K K I V N A K 30
31 K D V V N T K M F E E L K S R L D T L A Q E V A L L K E Q Q 60
61 A L Q T V C L K G T K V H M K C F L A F T Q T K T F H E A S 90
91 E D C I S R G G T L S T P Q T G S E N D A L Y E Y L R Q S V 120
121 G N E A E I W L G L N D M A A E G T W V D M T G A R I A Y K 150
151 N W E T E I T A Q P D G G K T E N C A V L S G A A N G K W F 180
181 D K R C R D Q L P Y I C Q F G I V * 197

Fig. 6



WO 98/56906

PCT/DK98/00245

7/21

SEQ ID NO: 82

TN12

G S I E G R G E P P
GATCCATCGAGGGTAGGGCGAGCCACCA ----- CAGACGGTCTA

SEQ ID NO:56

SEQ ID NO:83

Q T V *

SEQ ID NO:57

TN23

SEQ ID NO:84

G S I Q G R V V N T G I V *
GATCCATCCAGGGTAGGGTTGTGAACACA ----- GGGATCGTGT

SEQ ID NO:58

SEQ ID NO:85

SEQ ID NO:59

TN3

SEQ ID NO:86

G S I E G R A L Q G I V *
GATCCATCGAGGGTAGGGCCCTGCAG ----- GGGATCGTGT
SEQ ID NO:60

SEQ ID NO:85

SEQ ID NO:61

SEQ ID NO:87

M G S H H H H H H
CATATGGGATCGCATACCATACCACG ----- AGCTTGAAATT
SEQ ID NO:62 Bam HI Hind III Eco RI SEQ ID NO:63

Phage T7 promoter

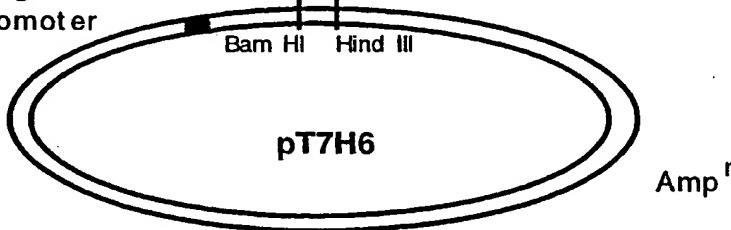


Fig. 7



WO 98/56906

PCT/DK98/00245

13/21

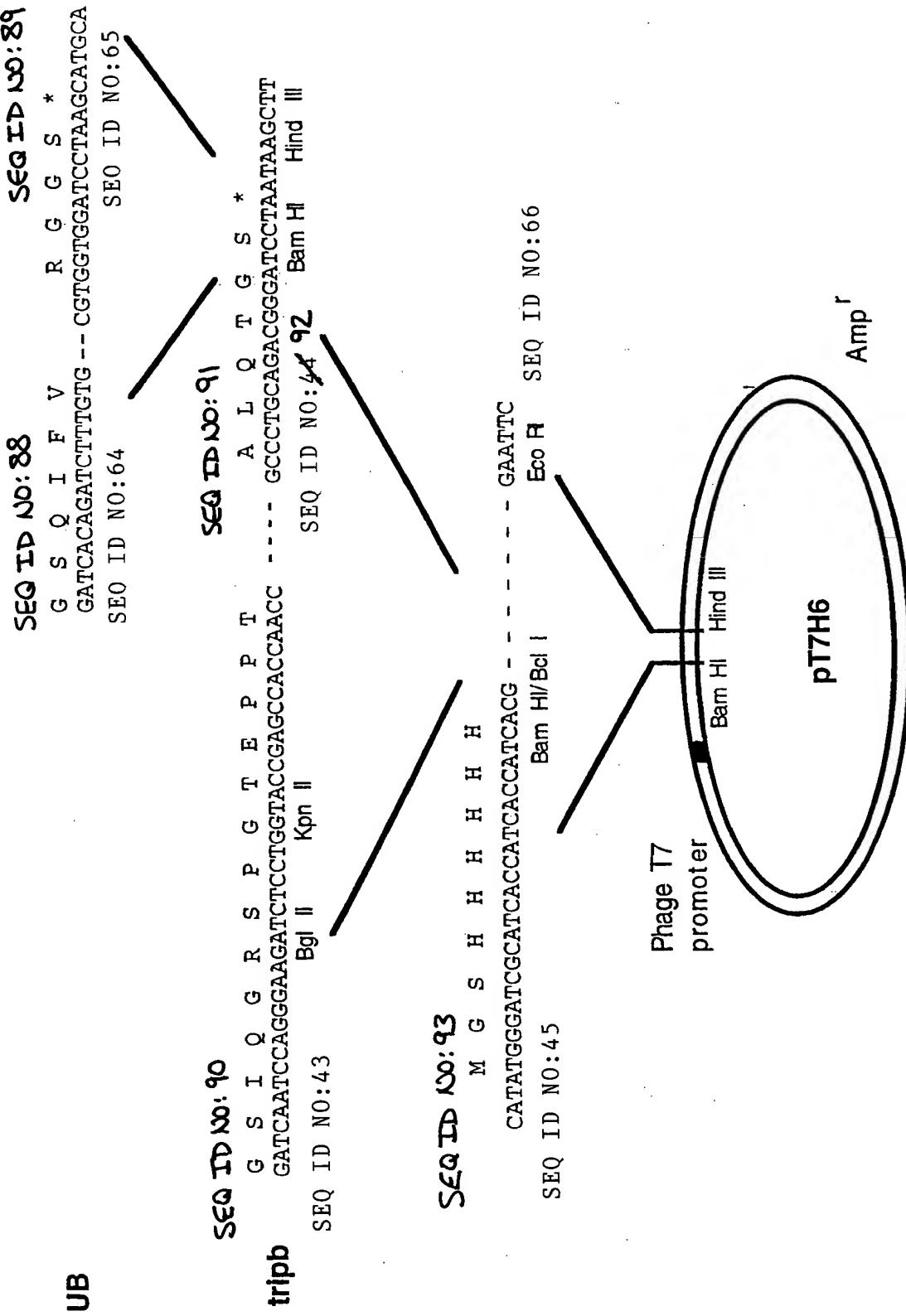


Fig. 13

Fig. 13



WO 98/56906

PCT/DK98/00245

15/21

pT7triplb

SEQ ID NO: 61

scFv(CEA6)

SEQ ID NO: 66

SEQ ID NO: 67

SEQ ID NO: 68

SEQ ID NO: 69

Phage T7 promoter

Bam HI

Eco RI

Amp^r

SEQ ID NO: 69

SEQ ID NO: 68

SEQ ID NO: 67

SEQ ID NO: 66

SEQ ID NO: 65

SEQ ID NO: 64

SEQ ID NO: 63

SEQ ID NO: 62

SEQ ID NO: 61

SEQ ID NO: 60

SEQ ID NO: 59

SEQ ID NO: 58

SEQ ID NO: 57

SEQ ID NO: 56

SEQ ID NO: 55

SEQ ID NO: 54

SEQ ID NO: 53

SEQ ID NO: 52

SEQ ID NO: 51

SEQ ID NO: 50

SEQ ID NO: 49

SEQ ID NO: 48

SEQ ID NO: 47

SEQ ID NO: 46

SEQ ID NO: 45

SEQ ID NO: 44

SEQ ID NO: 43

SEQ ID NO: 42

SEQ ID NO: 41

SEQ ID NO: 40

SEQ ID NO: 39

SEQ ID NO: 38

SEQ ID NO: 37

SEQ ID NO: 36

SEQ ID NO: 35

SEQ ID NO: 34

SEQ ID NO: 33

SEQ ID NO: 32

SEQ ID NO: 31

SEQ ID NO: 30

SEQ ID NO: 29

SEQ ID NO: 28

SEQ ID NO: 27

SEQ ID NO: 26

SEQ ID NO: 25

SEQ ID NO: 24

SEQ ID NO: 23

SEQ ID NO: 22

SEQ ID NO: 21

SEQ ID NO: 20

SEQ ID NO: 19

SEQ ID NO: 18

SEQ ID NO: 17

SEQ ID NO: 16

SEQ ID NO: 15

SEQ ID NO: 14

SEQ ID NO: 13

SEQ ID NO: 12

SEQ ID NO: 11

SEQ ID NO: 10

SEQ ID NO: 9

SEQ ID NO: 8

SEQ ID NO: 7

SEQ ID NO: 6

SEQ ID NO: 5

SEQ ID NO: 4

SEQ ID NO: 3

SEQ ID NO: 2

SEQ ID NO: 1

Fig. 15



WO 98/56906

PCT/DK98/00245

17/21

SEQ ID NO: 102
SEQ ID NO: 68
GATCCCCAGTTCACTG - - - CTCGAATGGGCCCTA
SEQ ID NO: 100

99
SEQ ID NO: 67
S Q V Q L N G A
GATCCCCAGTTCACTG - - - CTCGAATGGGCCCTA
SEQ ID NO: 67

SEQ ID NO: 102
Bam H Hind III
SEQ ID NO: 70

98
scFv(CEA6)
M G S H H H H G S I Q G R S P G T E P P T A L Q T G S *
CATATGGGATCGCATCACCATCACCCATCACGGATCAATTCCGGTACCGAGATCTCCGGTACCGAGGCCACCAACC--GCCCTGCAGACGGGATCCTAAAGCTTGAAATT
Bgl II Kpn II
Bam HI/Bcl I

SEQ ID NO: 101
Phage T7 promoter
Bam HI Eco RI
pT7tripp

SEQ ID NO: 69
Amp^r

Fig. 17



WO 98/56906

PCT/DK98/00245

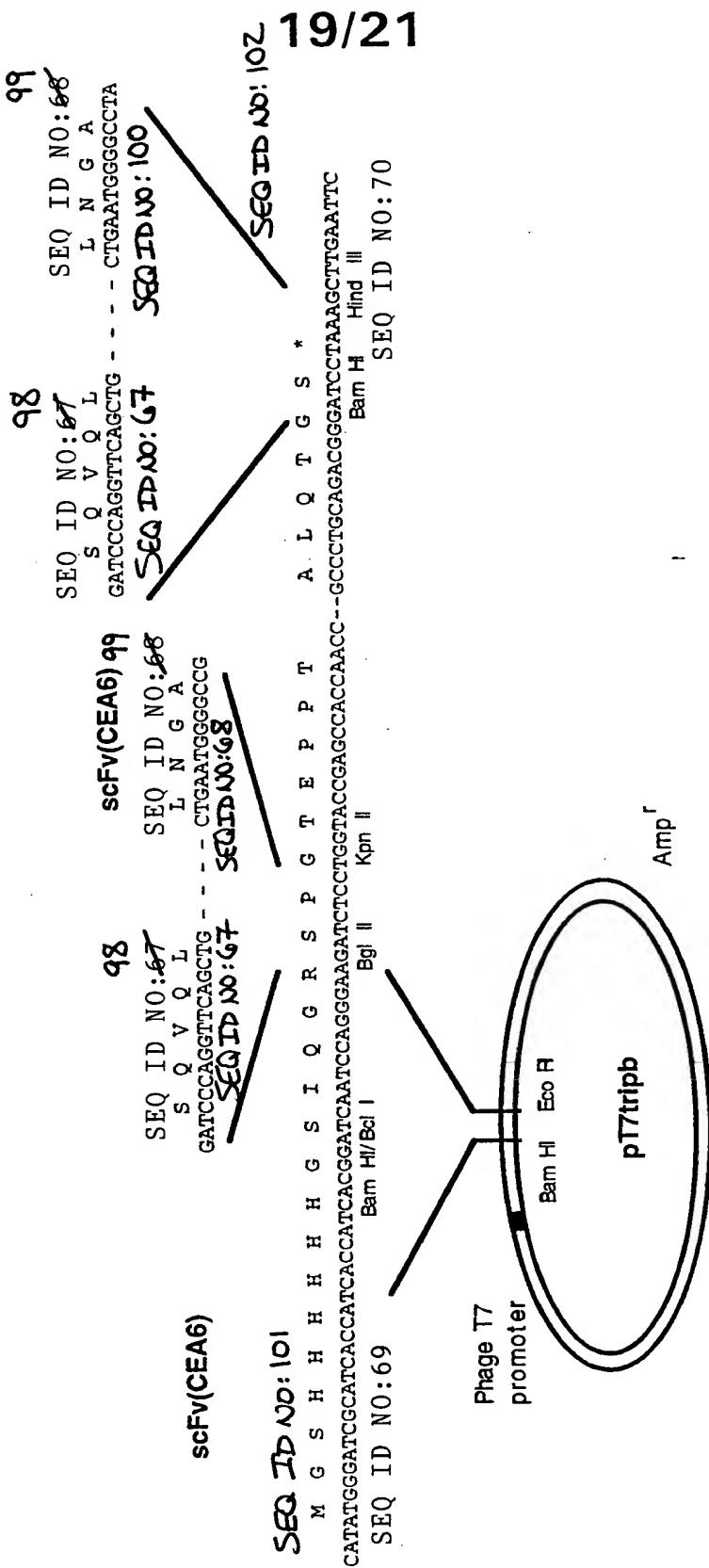
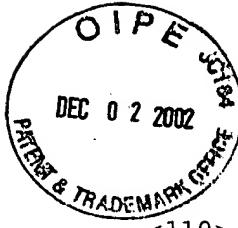


Fig. 19



SEQUENCE LISTING

<110> Borean Pharma A/S

<120> Trimerising module

<130> 62032.000004

<140> US 09/445,576

<141> 2000-07-17

<160> 104

<170> PatentIn version 3.1

<210> 1

<211> 47

<212> DNA

<213> Artificial

<220>

<223> primer trip-Ca

<400> 1

cctgatcaat ccagggaaaga ttcctggta ccgagccacc aacccag

47

D |<210> 2

<211> 33

<212> DNA

<213> Artificial

<220>

<223> primer trip-Ca

<400> 2

ccaagcttat taggatccc tctgcagggc ctg

33

<210> 3

<211> 40

<212> DNA

<213> Artificial

<220>

<223> trip-Cb

<400> 3

gcgaagctta ttaggatccc ttcaaggaga ccgtctgcag

40.

<210> 4

<211> 6

<212> PRT

<213> Artificial

<220>

<223> IQGR cleavage site

<400> 4

Gly Ser Ile Gln Gln Gly Arg
1 5

<210> 5
<211> 52
<212> PRT
<213> Artificial

<220>
<223> tetraneitin polypeptide sequence for Glu1 to Lys52

<400> 5

Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn Ala Lys Lys Asp
1 5 10 15

Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg Leu Asp Thr
20 25 30

Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala Leu Gln Thr
35 40 45

Val Ser Leu Lys
50

<210> 6
<211> 49
<212> PRT
<213> Artificial

<220>
<223> tetraneitin polypeptide sequence for Glu 1 to Val 49

<400> 6

Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn Ala Lys Lys Asp
1 5 10 15

Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg Leu Asp Thr
20 25 30

Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala Leu Gln Thr
35 40 45

Val

<210> 7
<211> 181
<212> PRT
<213> Artificial

<220>

<223> Mature tetranectin single chain

<400> 7

Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn Ala Lys Lys Asp
1 5 10 15

Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg Leu Asp Thr
20 25 30

Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala Leu Gln Thr
35 40 45

Val Cys Leu Lys Gly Thr Lys Val His Met Lys Cys Phe Leu Ala Phe
50 55 60

Thr Gln Thr Lys Thr Phe His Glu Ala Ser Glu Asp Cys Ile Ser Arg
65 70 75 80

Gly Gly Thr Leu Ser Thr Pro Gln Thr Gly Ser Glu Asn Asp Ala Leu
85 90 95

Tyr Glu Tyr Leu Arg Gln Ser Val Gly Asn Glu Ala Glu Ile Trp Leu
100 105 110

D/
W/ Gly Leu Asn Asp Met Ala Ala Glu Gly Thr Trp Val Asp Met Thr Gly
115 120 125

Ala Arg Ile Ala Tyr Lys Asn Trp Glu Thr Glu Ile Thr Ala Gln Pro
130 135 140

Asp Gly Gly Lys Thr Glu Asn Cys Ala Val Leu Ser Gly Ala Ala Asn
145 150 155 160

Gly Lys Trp Phe Asp Lys Arg Cys Arg Asp Gln Leu Pro Tyr Ile Cys
165 170 175

Gln Phe Gly Ile Val
180

<210> 8

<211> 39

<212> DNA

<213> Artificial

<220>

<223> Primer

<400> 8
cctggatcca tcgagggtag gggcgagcca ccaacccag

39

<210> 9
<211> 25
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 9
ccgaagctta cacgatcccc aactg

25

<210> 10
<211> 6
<212> PRT
<213> Artificial

<220>
<223> IEGR cleavage site

<400> 10

Gly Ser Ile Glu Gly Arg
1 5

<210> 11
<211> 32
<212> PRT
<213> Artificial

<220>
<223> lambda CII protein

<400> 11

Met Val Arg Ala Asn Lys Arg Asn Glu Ala Leu Arg Ile Glu Ser Ala
1 5 10 15

Leu Leu Asn Lys Ile Ala Met Leu Gly Thr Glu Lys Thr Ala Glu Gly
20 25 30

<210> 12
<211> 10
<212> PRT
<213> Homo sapiens

<400> 12

Gly Ser His His His His His Gly Ser
1 5 10

<210> 13
<211> 25

<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 13
ccgaagctta gaccgtctgc agggc 25

<210> 14
<211> 39
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 14
ggcgatcca tccaggtag gttgtgaac acaaagatg 39

<210> 15
<211> 36
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 15
cctggatcca tcgaggtag ggccctgcag acggc 36

D
<210> 16
<211> 227
<212> DNA
<213> Homo sapiens

<400> 16
atcgatct ttgtgaagac ctcactggc aaaaccatca cccttgaggt cgagccagt 60
gacaccattg agaatgtcaa agccaaatt caagacaagg aggttatccc acctgaccgc 120
agcgtctgat attgccccgc aaacagctgg aagatggacg tacttgtct gactacaata 180
ttcaaaaagga gtctactctt catcttgtgt tgagacttcg tggtggt 227

<210> 17
<211> 27
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 17
tgctgatcac agatcttgtt gaagacc 27

<210> 18
<211> 39
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 18
cgcaagcttg catgcttagg atccaccacg aagtctcaa

39

<210> 19
<211> 76
<212> PRT
<213> Homo sapiens

<400> 19

Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Thr Leu Glu
1 5 10 15

Val Glu Pro Ser Asp Thr Ile Glu Asn Val Lys Ala Lys Ile Gln Asp
20 25 30

Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys
35 40 45

Gln Leu Glu Asp Gly Arg Thr Leu Ser Asp Tyr Asn Ile Gln Lys Glu
50 55 60

Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly
65 70 75

<210> 20
<211> 786
<212> DNA
<213> Artificial

<220>
<223> CEA6 antibody

<400> 20

caggttcagc tgcagcagtc aggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60

tcctgcaagg cttctggagg caccttcagc aactctccta tcaactggct gcgacaggcc 120

cccgacaaag ggcttgagtg gatgggaagt atcatccctt cctttggtag agcaaactac 180

gctcagaagt tccaggccag actcacgatt accgcggacg aatccacgag cacagcctac 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gggcggagc 300

cacaactacg aactctacta ttactacatg gacgtctggg gccagggac aatggtcacc 360

gtctcgagtg gtggaggcgg ttcaggcgg a ggtggcagcg gcgggtggcgg atcggacatc 420

cagatgaccc agtctccttc caccctgtct gcatctattg gagacagagt caccatcacc 480
tgccgggcca gtgagggtat ttatcactgg ttggcctggg atcagcagaa gccagggaaa 540
gccctaaac tcctgatcta taaggcctct agtttagcca gtggggcccc atcaagggttc 600
agcggcagtg gatctggac agatttcaact ctcaccatca gcagcctgca gcctgatgat 660
tttgcaactt attactgcca acaatatagt aattatccgc tcactttcg cgagggacc 720
aagctggaga tcaaacgtgc ggccgcagaa caaaaactca tctcagaaga ggatctgaat 780
ggggcc 786

<210> 21
<211> 25
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 21
ggtgttatccc aggttcagct gcagc 25
ggtgttatccc aggttcagct gcagc

<210> 22
<211> 25
<212> DNA
<213> Artificial

D /
<220>
<223> Primer

<400> 22
gccggtacctg gccccattca gatcc 25
gccggtacctg gccccattca gatcc

<210> 23
<211> 26
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 23
tccaaagctta ggccccattc agatcc 26
tccaaagctta ggccccattc agatcc

<210> 24
<211> 228
<212> PRT
<213> Artificial

<220>
<223> CIIH6FXTN123

<400> 24

Met Val Arg Ala Asn Lys Arg Asn Glu Ala Leu Arg Ile Glu Ser Ala
1 5 10 15

Leu Leu Asn Lys Ile Ala Met Leu Gly Thr Glu Lys Thr Ala Glu Gly
20 25 30

Gly Ser His His His His His Gly Ser Ile Glu Gly Arg Gly Glu
35 40 45

Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn Ala Lys Lys Asp Val
50 55 60

Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg Leu Asp Thr Leu
65 70 75 80

Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala Leu Gln Thr Val
85 90 95

Cys Leu Lys Gly Thr Lys Val His Met Lys Cys Phe Leu Ala Phe Thr
100 105 110

Gln Thr Lys Thr Phe His Glu Ala Ser Glu Asp Cys Ile Ser Arg Gly
115 120 125

Dil
Gly Thr Leu Ser Thr Pro Gln Thr Gly Ser Glu Asn Asp Ala Leu Tyr
130 135 140

Glu Tyr Leu Arg Gln Ser Val Gly Asn Glu Ala Glu Ile Trp Leu Gly
145 150 155 160

Leu Asn Asp Met Ala Ala Glu Gly Thr Trp Val Asp Met Thr Gly Ala
165 170 175

Arg Ile Ala Tyr Lys Asn Trp Glu Thr Glu Ile Thr Ala Gln Pro Asp
180 185 190

Gly Gly Lys Thr Glu Asn Cys Ala Val Leu Ser Gly Ala Ala Asn Gly
195 200 205

Lys Trp Phe Asp Lys Arg Cys Arg Asp Gln Leu Pro Tyr Ile Cys Gln
210 215 220

Phe Gly Ile Val
225

<210> 25
<211> 197
<212> PRT
<213> Artificial

<220>
<223> H6FXTN123

<400> 25

Met Gly Ser His His His His His Gly Ser Ile Glu Gly Arg Gly
1 5 10 15

Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn Ala Lys Lys Asp
20 25 30

Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg Leu Asp Thr
35 40 45

Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala Leu Gln Thr
50 55 60

Val Cys Leu Lys Gly Thr Lys Val His Met Lys Cys Phe Leu Ala Phe
65 70 75 80

Thr Gln Thr Lys Thr Phe His Glu Ala Ser Glu Asp Cys Ile Ser Arg
85 90 95

Gly Gly Thr Leu Ser Thr Pro Gln Thr Gly Ser Glu Asn Asp Ala Leu
100 105 110

Tyr Glu Tyr Leu Arg Gln Ser Val Gly Asn Glu Ala Glu Ile Trp Leu
115 120 125

Gly Leu Asn Asp Met Ala Ala Glu Gly Thr Trp Val Asp Met Thr Gly
130 135 140

Ala Arg Ile Ala Tyr Lys Asn Trp Glu Thr Glu Ile Thr Ala Gln Pro
145 150 155 160

Asp Gly Gly Lys Thr Glu Asn Cys Ala Val Leu Ser Gly Ala Ala Asn
165 170 175

Gly Lys Trp Phe Asp Lys Arg Cys Arg Asp Gln Leu Pro Tyr Ile Cys
180 185 190

Gln Phe Gly Ile Val
195

<210> 26
<211> 65
<212> PRT
<213> Artificial

<220>
<223> H6FXTN12

<400> 26

Met Gly Ser His His His His His Gly Ser Ile Glu Gly Arg Gly
1 5 10 15

Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn Ala Lys Lys Asp
20 25 30

Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg Leu Asp Thr
35 40 45

Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala Leu Gln Thr
50 55 60

Val
65

<210> 27
<211> 180
<212> PRT
<213> Artificial

<220>
<223> H6FXTN23

<400> 27

Met Gly Ser His His His His His Gly Ser Ile Gln Gly Arg Val
1 5 10 15

Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg Leu Asp Thr Leu
20 25 30

Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala Leu Gln Thr Val
35 40 45

Cys Leu Lys Gly Thr Lys Val His Met Lys Cys Phe Leu Ala Phe Thr
50 55 60

Gln Thr Lys Thr Phe His Glu Ala Ser Glu Asp Cys Ile Ser Arg Gly
65 70 75 80

Gly Thr Leu Ser Thr Pro Gln Thr Gly Ser Glu Asn Asp Ala Leu Tyr
85 90 95

Glu Tyr Leu Arg Gln Ser Val Gly Asn Glu Ala Glu Ile Trp Leu Gly
100 105 110

Leu Asn Asp Met Ala Ala Glu Gly Thr Trp Val Asp Met Thr Gly Ala
115 120 125

Arg Ile Ala Tyr Lys Asn Trp Glu Thr Glu Ile Thr Ala Gln Pro Asp
130 135 140

Gly Gly Lys Thr Glu Asn Cys Ala Val Leu Ser Gly Ala Ala Asn Gly
145 150 155 160

Lys Trp Phe Asp Lys Arg Cys Arg Asp Gln Leu Pro Tyr Ile Cys Gln
165 170 175

Phe Gly Ile Val
180

<210> 28
<211> 73
<212> PRT
<213> Artificial

<220>
<223> H6FXtripa
<400> 28

Met Gly Ser His His His His His Gly Ser Ile Gln Gly Arg Ser
1 5 10 15

Pro Gly Thr Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn Ala
20 25 30

Lys Lys Asp Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg
35 40 45

Leu Asp Thr Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala
50 55 60

Leu Gln Thr Val Ser Leu Lys Gly Ser
65 70

<210> 29
<211> 69
<212> PRT

<213> Artificial

<220>

<223> H6FXtripb

<400> 29

Met Gly Ser His His His His His His Gly Ser Ile Gln Gly Arg Ser
1 5 10 15

Pro Gly Thr Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn Ala
20 25 30

Lys Lys Asp Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg
35 40 45

Leu Asp Thr Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala
50 55 60

Leu Gln Thr Gly Ser
65

<210> 30
<211> 152
<212> PRT
<213> Artificial

<220>
<223> H6FXTN3

<400> 30

Met Gly Ser His His His His His Gly Ser Ile Glu Gly Arg Ala
1 5 10 15

Leu Gln Thr Val Cys Leu Lys Gly Thr Lys Val His Met Lys Cys Phe
20 25 30

Leu Ala Phe Thr Gln Thr Lys Thr Phe His Glu Ala Ser Glu Asp Cys
35 40 45

Ile Ser Arg Gly Gly Thr Leu Ser Thr Pro Gln Thr Gly Ser Glu Asn
50 55 60

Asp Ala Leu Tyr Glu Tyr Leu Arg Gln Ser Val Gly Asn Glu Ala Glu
65 70 75 80

Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu Gly Thr Trp Val Asp
85 90 95

Met Thr Gly Ala Arg Ile Ala Tyr Lys Asn Trp Glu Thr Glu Ile Thr
100 105 110

Ala Gln Pro Asp Gly Gly Lys Thr Glu Asn Cys Ala Val Leu Ser Gly
115 120 125

Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys Arg Asp Gln Leu Pro
130 135 140

Tyr Ile Cys Gln Phe Gly Ile Val
145 150

<210> 31
<211> 145
<212> PRT
<213> Artificial

<220>
<223> H6FXtripb-UB

<400> 31

Met Gly Ser His His His His His Gly Ser Ile Gln Gly Arg Ser
1 5 10 15

Pro Gly Thr Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn Ala
20 25 30

Lys Lys Asp Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg
35 40 45

Leu Asp Thr Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala
50 55 60

Leu Gln Thr Gly Ser Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr
65 70 75 80

Ile Thr Leu Glu Val Glu Pro Ser Asp Thr Ile Glu Asn Val Lys Ala
85 90 95

Lys Ile Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile
100 105 110

Phe Ala Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu Ser Asp Tyr Asn
115 120 125

Ile Gln Lys Glu Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly
130 135 140

Ser
145

<210> 32
<211> 330
<212> PRT
<213> Artificial

<220>
<223> H6FXscFv(CEA6)-TRIPB

<400> 32

Met Gly Ser His His His His His Gly Ser Ile Gln Gly Arg Ser
1 5 10 15

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
20 25 30

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asn Ser
35 40 45

Pro Ile Asn Trp Leu Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
50 55 60

Gly Ser Ile Ile Pro Ser Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
65 70 75 80

Gln Gly Arg Leu Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
85 90 95

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
100 105 110

Ala Gly Arg Ser His Asn Tyr Glu Leu Tyr Tyr Tyr Met Asp Val
115 120 125

Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Gly Gly Gly Ser
130 135 140

Gly Gly Gly Ser Gly Gly Ser Asp Ile Gln Met Thr Gln
145 150 155 160

Ser Pro Ser Thr Leu Ser Ala Ser Ile Gly Asp Arg Val Thr Ile Thr
165 170 175

Cys Arg Ala Ser Glu Gly Ile Tyr His Trp Leu Ala Trp Tyr Gln Gln
180 185 190

Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Lys Ala Ser Ser Leu
195 200 205

Ala Ser Gly Ala Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
210 215 220

Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr
225 230 235 240

Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Leu Thr Phe Gly Gly Thr
245 250 255

Lys Leu Glu Ile Lys Arg Ala Ala Glu Gln Lys Leu Ile Ser Glu
260 265 270

Glu Asp Leu Asn Gly Ala Gly Thr Glu Pro Pro Thr Gln Lys Pro Lys
275 280 285

Lys Ile Val Asn Ala Lys Lys Asp Val Val Asn Thr Lys Met Phe Glu
290 295 300

Glu Leu Lys Ser Arg Leu Asp Thr Leu Ala Gln Glu Val Ala Leu Leu
305 310 315 320

D
Lys Glu Gln Gln Ala Leu Gln Thr Gly Ser
325 330

<210> 33
<211> 331
<212> PRT
<213> Artificial

<220>
<223> H6FXTRIPB-scFv(CEA6)

<400> 33

Met Gly Ser His His His His His Gly Ser Ile Gln Gly Arg Ser
1 5 10 15

Pro Gly Thr Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn Ala
20 25 30

Lys Lys Asp Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg
35 40 45

Leu Asp Thr Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala

50

55

60

Leu Gln Thr Gly Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val
65 70 75 80

Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly
85 90 95

Thr Phe Ser Asn Ser Pro Ile Asn Trp Leu Arg Gln Ala Pro Gly Gln
100 105 110

Gly Leu Glu Trp Met Gly Ser Ile Ile Pro Ser Phe Gly Thr Ala Asn
115 120 125

Tyr Ala Gln Lys Phe Gln Gly Arg Leu Thr Ile Thr Ala Asp Glu Ser
130 135 140

Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr
145 150 155 160

Ala Val Tyr Tyr Cys Ala Gly Arg Ser His Asn Tyr Glu Leu Tyr Tyr
165 170 175

Tyr Tyr Met Asp Val Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
180 185 190

Gly Gly Gly Ser Gly Gly Ser Gly Gly Ser Asp
195 200 205

Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Ile Gly Asp
210 215 220

Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Gly Ile Tyr His Trp Leu
225 230 235 240

Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
245 250 255

Lys Ala Ser Ser Leu Ala Ser Gly Ala Pro Ser Arg Phe Ser Gly Ser
260 265 270

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
275 280 285

Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Leu Thr
290 295 300

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Glu Gln
305 310 315 320

Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly Ala
325 330

<210> 34
<211> 592
<212> PRT
<213> Artificial

<220>
<223> H6FXscFv(CEA6)tripbscFv(CEA6)

<400> 34

Met Gly Ser His His His His His Gly Ser Ile Gln Gly Arg Ser
1 5 10 15

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
20 25 30

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asn Ser
35 40 45

Pro Ile Asn Trp Leu Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
50 55 60

Gly Ser Ile Ile Pro Ser Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
65 70 75 80

Gln Gly Arg Leu Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
85 90 95

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
100 105 110

Ala Gly Arg Ser His Asn Tyr Glu Leu Tyr Tyr Tyr Met Asp Val
115 120 125

Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Gly Gly Gly Ser
130 135 140

Gly Gly Gly Ser Gly Gly Ser Asp Ile Gln Met Thr Gln
145 150 155 160

Ser Pro Ser Thr Leu Ser Ala Ser Ile Gly Asp Arg Val Thr Ile Thr

165

170

175

Cys Arg Ala Ser Glu Gly Ile Tyr His Trp Leu Ala Trp Tyr Gln Gln
180 185 190

Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Lys Ala Ser Ser Leu
195 200 205

Ala Ser Gly Ala Pro Ser Arg Phe Ser Gly Ser Gly Thr Asp
210 215 220

Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr
225 230 235 240

Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Leu Thr Phe Gly Gly Thr
245 250 255

Lys Leu Glu Ile Lys Arg Ala Ala Glu Gln Lys Leu Ile Ser Glu
260 265 270

Glu Asp Leu Asn Gly Ala Gly Thr Glu Pro Pro Thr Gln Lys Pro Lys
275 280 285

Lys Ile Val Asn Ala Lys Asp Val Val Asn Thr Lys Met Phe Glu
290 295 300

Glu Leu Lys Ser Arg Leu Asp Thr Leu Ala Gln Glu Val Ala Leu Leu
305 310 315 320

Lys Glu Gln Gln Ala Leu Gln Thr Gly Ser Gln Val Gln Leu Gln Gln
325 330 335

Ser Gly Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys
340 345 350

Lys Ala Ser Gly Gly Thr Phe Ser Asn Ser Pro Ile Asn Trp Leu Arg
355 360 365

Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Ser Ile Ile Pro Ser
370 375 380

Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe Gln Gly Arg Leu Thr Ile
385 390 395 400

Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu
405 410 415

Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Gly Arg Ser His Asn
420 425 430

Tyr Glu Leu Tyr Tyr Tyr Met Asp Val Trp Gly Gln Gly Thr Met
435 440 445

Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly
450 455 460

Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser
465 470 475 480

Ala Ser Ile Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Gly
485 490 495

Ile Tyr His Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
500 505 510

Lys Leu Leu Ile Tyr Lys Ala Ser Ser Leu Ala Ser Gly Ala Pro Ser
515 520 525

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
530 535 540

Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser
545 550 555 560

Asn Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
565 570 575

Ala Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly Ala
580 585 590

<210> 35
<211> 51
<212> PRT
<213> Homo sapiens

<400> 35

Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn Ala Lys Lys Asp
1 5 10 15

Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg Leu Asp Thr
20 25 30

Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala Leu Gln Thr
35 40 45

Val Cys Leu
50

<210> 36
<211> 36
<212> PRT
<213> Homo sapiens

<400> 36

Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg Leu Asp Thr
1 5 10 15

Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala Leu Gln Thr
20 25 30

Val Cys Leu Lys
35

<210> 37
<211> 36
<212> PRT
<213> Murine

<400> 37

D/V
Leu Val Ser Ser Lys Met Phe Glu Glu Leu Lys Asn Arg Met Asp Val
1 5 10 15

Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Lys Gln Ala Leu Gln Thr
20 25 30

Val Cys Leu Lys
35

<210> 38
<211> 36
<212> PRT
<213> Bovine

<400> 38

Arg Arg Val Lys Glu Lys Asp Gly Asp Leu Lys Thr Gln Val Glu Lys
1 5 10 15

Leu Trp Arg Glu Val Asn Ala Leu Lys Glu Met Gln Ala Leu Gln Thr
20 25 30

Val Cys Leu Arg
35

<210> 39
<211> 36
<212> PRT
<213> Shark

<400> 39

Ser Lys Ser Gly Lys Gly Lys Asp Asp Leu Arg Asn Glu Ile Asp Lys
1 5 10 15

Leu Trp Arg Glu Val Asn Ser Leu Lys Glu Met Gln Ala Leu Gln Thr
20 25 30

Val Cys Leu Lys
35

<210> 40
<211> 36
<212> PRT
<213> Artificial

<220>
<223> Consencus sequence

<220>
<221> MISC_FEATURE
<222> (1)...(9)
<223> variable

D/
D/
<220>
<221> MISC_FEATURE
<222> (11)...(16)
<223> variable

<220>
<221> MISC_FEATURE
<222> (18)...(19)
<223> variable

<220>
<221> MISC_FEATURE
<222> (22)...(23)
<223> variable

<220>
<221> MISC_FEATURE
<222> (27)...(27)
<223> variable

<220>
<221> MISC_FEATURE
<222> (36)..(36)
<223> variable

<400> 40

Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Leu Xaa Xaa Glu Val Xaa Xaa Leu Lys Glu Xaa Gln Ala Leu Gln Thr
20 25 30

Val Cys Leu Xaa
35

<210> 41
<211> 41
<212> DNA
<213> Artificial

<220>
<223> tripa

<400> 41
gatcaatcca gggaaagatct cctggtaaccg agccaccaac c 41

D/W
<210> 42
<211> 24
<212> DNA
<213> Artificial

<220>
<223> tripa

<400> 42
acgttctccc tgaaggatc ctaa 24

<210> 43
<211> 41
<212> DNA
<213> Artificial

<220>
<223> tripb

<400> 43
gatcaatcca gggaaagatct cctggtaaccg agccaccaac c 41

<210> 44
<211> 21
<212> DNA
<213> Artificial

<220>
<223> tripb

<400> 44
gccctgcaga cgggatccta a 21

<210> 45
<211> 31
<212> DNA
<213> Artificial

<220>
<223> pT7H6

<400> 45
catatggat cgcatcacca tcaccatcac g 31

<210> 46
<211> 11
<212> DNA
<213> Artificial

<220>
<223> pT7H6

<400> 46
agcttgaatt c 11

<210> 47
<211> 26
<212> DNA
<213> Artificial

<220>
<223> TN123

<400> 47
gatccatcga ggtaggggc gagcca 26

<210> 48
<211> 8
<212> DNA
<213> Artificial

<220>
<223> tn123

<400> 48
atcgtgta 8

<210> 49
<211> 15
<212> DNA
<213> Artificial

<220>

<223> pT7CIIH6

<400> 49

catatggttc gtgca

15

<210> 50

<211> 31

<212> DNA

<213> Artificial

<220>

<223> pT7CIIH6

<400> 50

gaagggggat cgcatcacca tcaccatcac g

31

<210> 51

<211> 11

<212> DNA

<213> Artificial

<220>

<223> pT7CIIH6

<400> 51

agcttgaatt c

11

<210> 52

<211> 26

<212> DNA

<213> Artificial

<220>

<223> TN123

<400> 52

gatccatcga gggtaggggc gagcca

26

<210> 53

<211> 8

<212> DNA

<213> Artificial

<220>

<223> TN123

<400> 53

atcggtgta

8

<210> 54

<211> 31

<212> DNA

<213> Artificial

<220>

<223> pT7H6

<400> 54
catatgggat cgccatcacca tcaccatcac g 31

<210> 55
<211> 11
<212> DNA
<213> Artificial

<220>
<223> pT7H6

<400> 55
agcttgaatt c 11

<210> 56
<211> 29
<212> DNA
<213> Artificial

<220>
<223> TN12

<400> 56
gatccatcga ggtaggggc gagccacca 29

<210> 57
<211> 11
<212> DNA
<213> Artificial

D
<220>
<223> TN12

D
<400> 57
cagacgggtct a 11

<210> 58
<211> 29
<212> DNA
<213> Artificial

<220>
<223> TN23

<400> 58
gatccatcca ggtagggtt gtgaacaca 29

<210> 59
<211> 11
<212> DNA
<213> Artificial

<220>
<223> TN23

<400> 59
gggatcgtgt a

11

<210> 60
<211> 26
<212> DNA
<213> Artificial

<220>
<223> TN3

<400> 60
gatccatcga gggtagggcc ctgcag

26

<210> 61
<211> 11
<212> DNA
<213> Artificial

<220>
<223> TN3

<400> 61
gggatcgtgt a

11

<210> 62
<211> 31
<212> DNA
<213> Artificial

<220>
<223> pT7H6

<400> 62
catatggat cgcacacca tcaccatcac g

31

<210> 63
<211> 11
<212> DNA
<213> Artificial

<220>
<223> pT7H6

<400> 63
agcttgaatt c

11

<210> 64
<211> 17
<212> DNA
<213> Artificial

<220>
<223> UB

<400> 64

gatcacagat ctttgtg

17

<210> 65
<211> 22
<212> DNA
<213> Artificial

<220>
<223> UB

<400> 65
cgtggatggat cctaaggcatg ca

22

<210> 66
<211> 6
<212> DNA
<213> Artificial

<220>
<223> pT7H6

<400> 66
gaattc

6

<210> 67
<211> 17
<212> DNA
<213> Artificial

<220>
<223> scFv(CEA6)

<400> 67
gatcccaggt tcagctg

17

<210> 68
<211> 13
<212> DNA
<213> Artificial

<220>
<223> scFv(CEA6)

<400> 68
ctgaatgggg ccg

13

<210> 69
<211> 72
<212> DNA
<213> Artificial

<220>
<223> pT7tribb

<400> 69
catatggatcgcatcacca tcaccatcac ggatcaatcc aggaaagatc tcctggta

60

gagccaccaa cc

72

<210> 70
<211> 32
<212> DNA
<213> Artificial

<220>
<223> pT7tripb

<400> 70
gcctgcaga cgggatccta aagcttgaat tc

32

<210> 71
<211> 52
<212> PRT
<213> Homo sapiens

<400> 71

Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn Ala Lys Lys Asp
1 5 10 15

Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg Leu Asp Thr
20 25 30

Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala Leu Gln Thr
35 40 45

D) Val Cys Leu Lys
50

<210> 72
<211> 14
<212> PRT
<213> Artificial

<220>
<223> tripa

<400> 72

Gly Ser Ile Gln Gly Arg Ser Pro Gly Thr Glu Pro Pro Thr
1 5 10

<210> 73
<211> 7
<212> PRT
<213> Artificial

<220>
<223> tripa

<400> 73

Thr Val Ser Leu Lys Gly Ser
1 5

<210> 74

<211> 14

<212> PRT

<213> Artificial

<220>

<223> tripb

<400> 74

Gly Ser Ile Gln Gly Arg Ser Pro Gly Thr Glu Pro Pro Thr
1 5 10

<210> 75

<211> 6

<212> PRT

<213> Artificial

<220>

<223> tripb

<400> 75

Ala Leu Gln Thr Gly Ser
1 5

D) <210> 76

<211> 9

<212> PRT

<213> Artificial

<220>

<223> pT7H6

<400> 76

Met Gly Ser His His His His His His
1 5

<210> 77

<211> 9

<212> PRT

<213> Artificial

<220>

<223> pT7CIIH6

<400> 77

Gly Ser Ile Glu Gly Arg Gly Glu Pro
1 5

<210> 78
<211> 2
<212> PRT
<213> Artificial

<220>
<223> pT7CIIH6

<400> 78

Ile Val
1

<210> 79
<211> 4
<212> PRT
<213> Artificial

<220>
<223> pT7CIIH6

<400> 79

Met Val Arg Ala
1

<210> 80
<211> 10
<212> PRT
<213> Artificial

<220>
<223> pT7CIIH6

<400> 80

DIV Glu Gly Gly Ser His His His His His
1 5 10

<210> 81
<211> 9
<212> PRT
<213> Artificial

<220>
<223> pT7CIIH6

<400> 81

Met Gly Ser His His His His His His
1 5

<210> 82
<211> 10
<212> PRT
<213> Artificial

<220>
<223> pT7H6

<400> 82

Gly Ser Ile Glu Gly Arg Gly Glu Pro Pro
1 5 10

<210> 83
<211> 3
<212> PRT
<213> Artificial

<220>
<223> pT7H6

<400> 83

Gln Thr Val
1

<210> 84
<211> 10
<212> PRT
<213> Artificial

<220>
<223> pT7H6

<400> 84

Gly Ser Ile Gln Gly Arg Val Val Asn Thr
1 5 10

<210> 85
<211> 3
<212> PRT
<213> Artificial

<220>
<223> pT7H6

<400> 85

Gly Ile Val
1

<210> 86
<211> 9
<212> PRT
<213> Artificial

<220>
<223> pT7H6

<400> 86

Gly Ser Ile Glu Gly Arg Ala Leu Gln
1 5

<210> 87
<211> 9
<212> PRT
<213> Artificial

<220>
<223> pT7H6

<400> 87

Met Gly Ser His His His His His His
1 5

<210> 88
<211> 6
<212> PRT
<213> Artificial

<220>
<223> pT7H6

<400> 88

Gly Ser Gln Ile Phe Val
1 5

<210> 89
<211> 4
<212> PRT
<213> Artificial

<220>
<223> pT7H6

<400> 89

Arg Gly Gly Ser
1

<210> 90
<211> 14
<212> PRT
<213> Artificial

<220>
<223> pT7H6

<400> 90

Gly Ser Ile Gln Gly Arg Ser Pro Gly Thr Glu Pro Pro Thr
1 5 10

<210> 91
<211> 6
<212> PRT
<213> Artificial

<220>
<223> pT7H6

<400> 91

Ala Leu Gln Thr Gly Ser
1 5

<210> 92
<211> 28
<212> DNA
<213> Artificial

<220>
<223> pT7H6

<400> 92
gccctgcaga cgggatccta ataaagctt

28

<210> 93
<211> 9
<212> PRT
<213> Artificial

<220>
<223> pT7H6

<400> 93

Met Gly Ser His His His His His His
1 5

<210> 94
<211> 5
<212> PRT
<213> Artificial

<220>
<223> pT7tripb

<400> 94

Ser Gln Val Gln Leu
1 5

<210> 95
<211> 4
<212> PRT
<213> Artificial

<220>
<223> pT7tripb

<400> 95

Leu Asn Gly Ala
1

<210> 96
<211> 23
<212> PRT
<213> Artificial

<220>
<223> pT7tripb

<400> 96

Met Gly Ser His His His His His Gly Ser Ile Gln Gly Arg Ser
1 5 10 15

Pro Gly Thr Glu Pro Pro Thr
20

<210> 97
<211> 6
<212> PRT
<213> Artificial

<220>
<223> pT7tripb

<400> 97

Ala Leu Gln Thr Gly Ser
1 5

D
<210> 98
<211> 5
<212> PRT
<213> Artificial

<220>
<223> pT7tripb

<400> 98

Ser Gln Val Gln Leu
1 5

<210> 99
<211> 4
<212> PRT
<213> Artificial

<220>
<223> pT7tripb

<400> 99

Leu Asn Gly Ala
1

<210> 100
<211> 14
<212> DNA
<213> Artificial

<220>
<223> pT7tripb

<400> 100
ctgaatgggg ccta

14

<210> 101
<211> 23
<212> PRT
<213> Artificial

<220>
<223> pT7tripb

<400> 101

Met Gly Ser His His His His His Gly Ser Ile Gln Gly Arg Ser
1 5 10 15

Pro) Gly Thr Glu Pro Pro Thr
20

DW
<210> 102
<211> 6
<212> PRT
<213> Artificial

<220>
<223> pT7tripb

<400> 102

Ala Leu Gln Thr Gly Ser
1 5

<210> 103
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> FXa Cleavage site

<400> 103

Ile Gln Gly Arg

1

<210> 104
<211> 4
<212> PRT
<213> Artificial Sequence

> <220>
<223> FXa protease.site

<400> 104

Ile Glu Gly Arg

1